APPENDIX

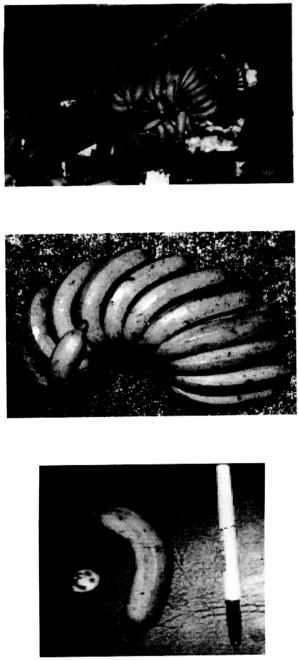


Plate 1. RI (*Musa acuminata ssp. malaccensis*) showing degenerated male bud and rachis, fruits curved and bottle necked.

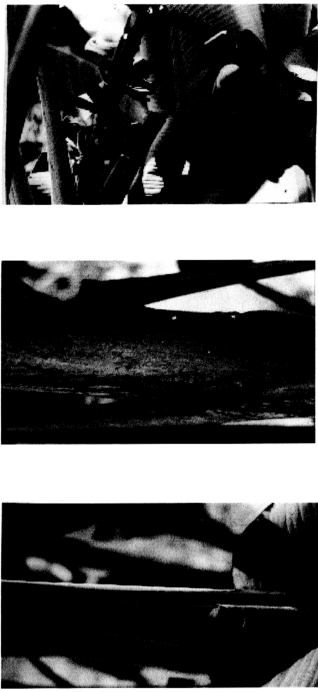


Plate 2. IPTJ (*Musa acuminata ssp. malaccensis*) male bud color red and convolute, pseudostem green very waxy, round asymmetrical lamina bases, petioles very waxy.

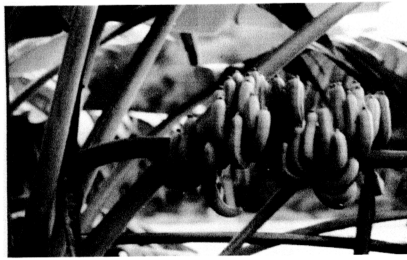
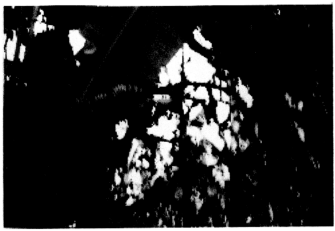


Plate 3. BD1 (*Musa acuminata ssp. malaccensis*) characterized with short stature, red color bract with yellow streaks, horizontal fruit bunch, rachis very hairy.



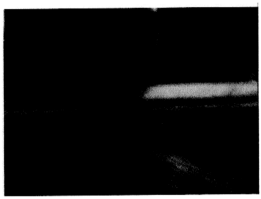


Plate 4. BD2 (*Musa acuminata ssp. malaccensis*) a tall banana with horizontal fruit bunch and rachis, leaf base rounded, petioles and leaves very waxy.

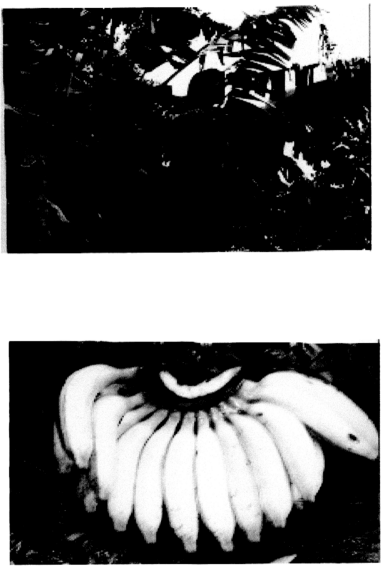


Plate 5. BC1 (*Musa acuminata ssp. malaccensis*) a tall banana collected from Genting foothills showing big horizontal fruit bunch with large hands and fingers.

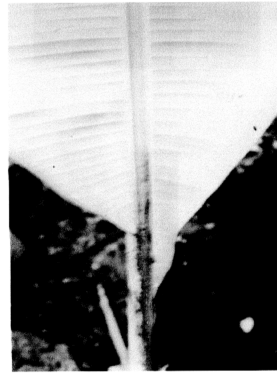


Plate 6. BC3 (*Musa acuminata ssp. truncata*) a highland banana collected from 2000m altitude characterized with deep chocolate brown color of pseudostem, petioles and male bud. Both lamina bases are pointed with non-waxy petioles and leaves. Creamy color of the bract internal face.

Plate 6 continued: sample BC3

Plate 7. Flava (*Musa acuminata ssp. malaccensis*) a yellow bracted mutant, with slightly imbricated male bracts, yellowish green color of the pseudostem with black blotches. Fruit bunch and rachis horizontal with small fruit bunch.

Plate 8. Segun (*Musa acuminata ssp. malaccensis*) characterized with very lax fruit bunch, slight imbricated male bud, petiole bases symmetrical and petiole bases very waxy.

Plate 9. Rangis (*Musa acuminata ssp. malaccensis*) male bracts greatly imbricated with yellow color on the bract apex.

Plate 10. Kra (*Musa acuminata ssp. malaccensis*) showing red color of the male bract, rachis hanging at an angle, fruit bunch compact.

Plate 11. PPC (*Musa acuminata ssp. malaccensis*) characterized with yellowish green color of pseudostem and petiole canal waxy.

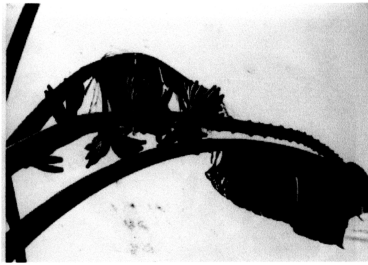


Plate 12. Sintok (*Musa acuminata ssp. malaccensis*) showing horizontal fruit bunch and rachis, fruit bunch very lax, rachis glabrous and petiole bases waxy.

Plate 13. Perak (*Musa acuminata ssp. malaccensis*) showing horizontal fruit bunch with few fruits, fruits with male flowers persistent, petiole canal wide with erect margins.

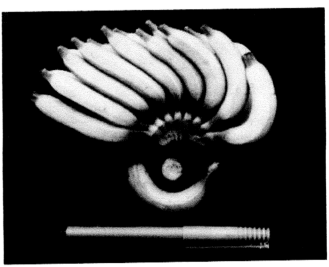


Plate 14. J-4 (*Musa acuminata ssp. malaccensis*) showed yellowish green color of pseudostem with brown blotches, small horizontal fruit bunch, fruits bottle necked and male bracts convolute.

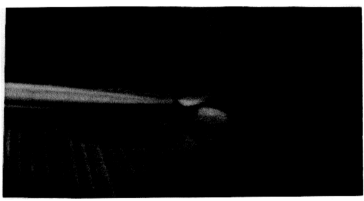


Plate 15. Gala (*Musa balbisiana*) characterized with imbricated male bud, bracts lifting two or more at a time and non - revolute, rachis hanging at an angle, petiole canal closed, fruit bunch very compact, fruits dull in appearance and blunt tipped.

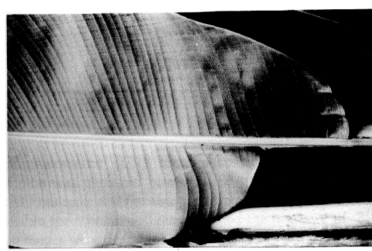


Plate 16. BC2 (*Musa violascens*) erect fruit rachis, pink color of male bracts, fruits blunt tipped, fruit bunch very compact and male bracts very imbricated, leaf bases asymmetrical.

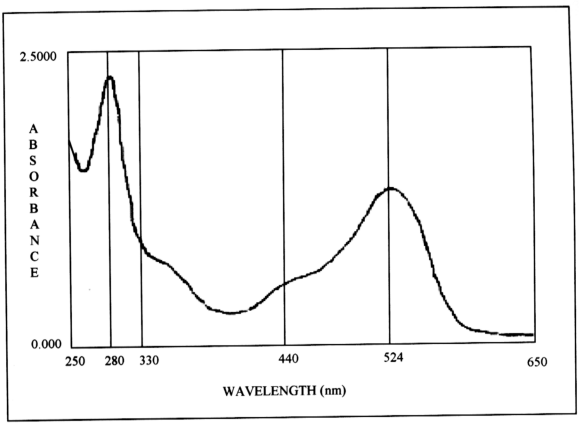


Plate 17. Spectrophotometric (UV) absorbance analysis of anthocyanins. The maximum absorbance was observed between 280 and 524 nm. The absence of a shoulder around 330 nm indicated the absence of acylation whereas the presence of a shoulder in the visible spectrum at 440 nm indicated the presence of 3 – rutinosides. Similar absorption ranges were described by Horry and Jay (1988a).

Appendix 2.

MURASHIGE AND SKOOG MEDIA (1962)

Macro Stock Solution	**2L Stock (Use: 100ml/L)**
Ammonium nitrate (NH_4NO_3)	33.0 g
Potassium Nitrate (KNO_3)	38.0 g
Calcium Chloride ($CaCl_2.2H_2O$)	8.8 g
Magnesium Sulphate ($MgSO_4.7H_2O$)	7.4 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	3.4 g
Micro Stock Solution	**2L Stock (Use: 10ml/L)**
Manganese Sulphate ($MnSO_4.4H_2O$)	4.46 g
Zinc Sulphate ($ZnSO_4.H_2O$)	1.72 g
Potassium Iodide (KI)	0.17 g
Cupric Sulphate ($CuSO_4.5H_2O$)	0.0052 g
Sodium Molybdate ($Na_2MoO_4.2H_2O$)	0.05 g
Cobaltus Chloride ($CoCl_2.6H_2O$)	0.0052 g
Boric acid (H_3BO_3)	1.24 g
Vitamin Stock Solution	**500ml Stock (Use: 2ml/L)**
Nicotinic acid	0.125 g
Thiamine HCl	0.25 g
Pyridoxine HCl	0.125 g
Myo-Inositol	25.0 g
Glycine	0.50 g
Fe Source Stock Solution	**500ml Stock (Use: 5ml/L)**
Sodium EDTA Ferric Salt	3.75 g
Ferrous sulphate	2.78 g
Others	
Sucrose	40.0 g/L
Gelrite	1.75 g/L
pH : 5.8	

Appendix 3.

***Knudson's Medium* (1950)**

Basal Medium

Calcium nitrate tetrahydrate $Ca(NO_3)_2 . 4H_2O$	1.0g/L
Pottasium dihydrogen orthophosphate (KH_2PO_4)	0.25g/L
Ammonium sulphate $(NH_4)_2 . SO_4$	0.50g/L
Ferrum phosphate tetrahydrate ($Fe PO_4 . 4H_2O$)	0.25g/L

Appendix 4.

Composition of the lysis buffer LB01 (Dolezel et al., 1989)

Following components were dissolved in deionized water;

1. 363 mg of Tris (15mM)
2. 148.9 mg of Na_2EDTA (2mM)
3. 20.2 mg Spermine (0.05mM)
4. 1.193 g KCl (80mM)
5. 233.8mg NaCl (20mM)
6. 200µl Triton X-100 (0.1%).

The final volume was adjusted to 200µl with deionized water, pH was adjusted to 7.5 by using 1N NaOH. Buffer was filtered through a 0.22µm filter to remove small debris particles. Finally 220µl of mercaptoethanol was added to a final concentration of 15mM and was mixed thoroughly. Buffer was aliquot in 10ml, stored at -20^{O}C.

Appendix 5.

Preparation of DAPI Stock Solution

DAPI was dissolved in deionized water and stock solutions were prepared as followed;

1. 5mg of DAPI (4,6 - diamidino - 2 - phenylindole) was dissolved to a final volume of 50ml.
2. Solution was filtered through a 0.22μm filter to remove small debris particles.
3. Finally mixture was aliquot in 1ml and stored at -20^{O}C.

The optimal final concentration of DAPI for staining of isolated nuclei depends on many factors. However, satisfactory results were obtained with 2μg/ml. 200μl DAPI was added to LB01 solution before chopping of the tissue. Tissue was chopped in 1ml of lysis buffer. Then the homogenate was filtered through a nylon filter (50μm pore size), to remove the leaf tissue debris.

Appendix 6.

Propidium Iodide (PI) Staining For DNA Content

Add 2.9 μl of a Ribonuclease A stock solution (24 mg/ml) and 10μl of PI stock solution (10 mg/ml). For both procedures, either 2% of PVP (polyvinylpyelidone) in final concentration or mercaptoethanol (final 15 mM) to the lysis buffers were also used.

Appendix 7.

Preparation of Chicken erythrocyte buffer for FCM Calibration

a, Buffer I (Dissolve in deionized H_2O)

Na Cl	1.637 g
$Na_3C_6H_5O_7.2H_2O$	588.2 mg
Tris	24.23 mg

Adjust volume to 200 ml with deionized water and pH = 7.1

b, Buffer II (Dissolve in deionized H_2O)

NaCl	0.41 g
Triton X - 100	2.5 ml

Adjust volume to 50 ml with deionized H_2O.

C, Buffer III (Dissolve in deionized H_2O)

Sucrose	54.77 g
$Mg\ SO_4.\ 2H_2O$	1.856 mg
Tris	60.57 mg
Mercaptoethanol	20 µl

Adjust to volume of 500 ml with deionized H_2O and pH = 7.1

CURRICULUM VITAE

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Qualifications:

Ph.D. Plant Breeding, University of Malaya, Kuala Lumpur, Malaysia (2001)

M. Sc. (Hons). Plant Breeding, University of Agriculture Faisalabad, Pakistan (1992-1995)

B. Sc. (Hons). Plant Breeding, University of Agriculture Faisalabad, Pakistan (1987-1991)

Employment:

1. Research Assistant in Division of Genetics and Cellular Biology (ISB), University of Malaya (1996-1999, 2001-). As a research assistant I was engaged in the following research projects.

- In vitro propagation of different cultivated bananas (Berangan, Mas, Nipah, Cinta Manis, Rastali, Serendah, Novaria, Raja Udang) using suckers as explant.

- In vitro propagation of cultivated bananas by using male buds (Berangan,Mas, Cina Manis, Nipah and Musa acuminata ssp. malaccensis)

- Micropropagation of strawberry, chrysanthemum, Canna, and Watermelon.

- Mutation breeding studies in Berangan and field evaluation.

- Somaclonal studies in Berangan and Raja Udang.

- Banana collection trip to Sarawak (1996)

- Trained foreign researchers from Sudan, Somalia, India, Indonesia,Bangladesh and Philippine in banana micropropagation, Fusarium wilt screening and DNA fingerprinting.

- Assisted 9 undergraduate and 3 M. Sc projects.

2. Lab. Manager in Banana Tissue Culture Commercial Unit, Institute of Biological Sciences (ISB), University of Malaya (1999-2000).

- Commercial production of Berangan, Mas, Rastali and Cinta Manis.

- Utilization of bar coding system for recording and monitoring banana cultures.

3.Demonstrator in National Workshop on " In vitro Plant Multiplication, Selection, Mutagenesis, and Molecular Marker Studies in Plant Improvement" held at MINT Technology Park, Jalan Dengkil (29^{th} October – 9^{th} November 2001).

Training

1- Utilization of Flow cytometry (FCM) for ploidy and genomic DNA content determination in banana. Organized by Malaysian Institute of Nuclear Technology (MINT) Bangi and International Atomic Energy Agency (IAEA)1997.

2- Molecular techniques in Banana (RAPD, RAMPO). Organized by Malaysian Institute of Nuclear Technology (MINT) Bangi and International Atomic Energy Agency (IAEA) 1998.

3- Somatic embryogenesis in Bananas (Initiation of scalps and cell suspensions). One week training program held at Institute of Biological Sciences, University of Malaya (UM) 1998.

4- AFLP (Amplified Fragment Length Polymorphism) DNA markers training in RRI (Rubber Research Institute) Sungei Buloh 1998.

5- Fusarium wilt isolation, inoculation and characterization. Two weeks training program at University of Sains Malaysia (USM) Penang 1999.

Seminars & Workshops

1- Second FAO/IAEA Coordinated Research Meeting (13-17 October, 1997) Kuala Lumpur, Malaysia.

2- The first National Banana Seminar (23-25 November, 1998) Awana Genting,

3- Pahang.Bengkel Pengendalian Pisang Dari Makmal ke Ladang 8-12 Mei, 2000. Institute of Biological Sciences (ISB), University of Malaya.

4- Tropical Plant Biological Research in Malaysia: Fruits and Vegetables. (4-7 September 2000) Terengganu.

5- Banana Biotechnology Workshop, (2-5 July, 2001), Rimba Ilmu, University of Malaya.

Membership

INIBAP (International Program for the Improvement of Banana and Plantains Program)

Scholarship

Ciba Geighy M.Sc (Hons) Merit scholarship

Posters & Publications:

1. Asif, M. J., (1995). Correlation and Path - Coefficient analysis of yield and yield components in Groundnut (Arachis hypogaea L). M. Sc thesis submitted to the Faculty of Agriculture University of Agriculture Faisalabad, Pakistan.

2. Asif, M. J., Mak, C. and R. Y. Othman, (1997). RAPD marker studies in wild Musa species. Poster presented at Second Research Coordination Meeting of FAO/IAEA/BADC Co-ordinated Research Project held in Kuala Lumpur, Malaysia, 13-17 October, Cellular biology and Biotechnology including Mutation Techniques for Creation of new useful Banana Genotypes.

3. Asif, M. J., Mak, C. and R. Y. Othman, (1998). In vitro zygotic embryo culture studies in wild Musa species and factors affecting the growth of the seedlings. Poster presented at first National Banana Seminar, Awana Genting, Malaysia, 13-15 January, 1998,

4. Mak, C., Ho, Y. H., Liew, K. W., Azhar, M., Asif, M. J. and A. A.Mohammed, (1999). In vitro manipulation and mutation breeding for the improvement of banana. Paper presented at 3rd FAO/IAEA Research Coordination meeting of the Collaborative Research Project "Cellular Biology and Biotechnology including mutation Techniques for the Creation of new Useful Banana genotypes" held in Sri Lanka, InfoMusa, Vol. 8 (2).

5. Yasmin, R. Y., Asif, M. J., Eswari, T., Uthayubanu, G. and A. L. Lim, (1999). Banana Streak Virus Detection in Malaysian Bananas. In: Proceedings of the 5th International Conference of Plant Protection in the Tropics (Use of PCR Technology).

6. Asif, M. J., Mak, C. and R. Y. Othman, (2000). Polyploidy induction in a local wild banana (Musa acuminata ssp. malaccensis). Pakistan Journal of Biological Sciences 3(5): 740-743.

7. Othman, R. Y., Khalid, N., Asif, M. J., Mak, C. and S. H. Tan, (2000). Molecular Studies on Musa acuminata ssp. malaccensis and selected local Malaysian species. In: Recent advances in the molecular and cellular biology of Bananas. Paper presented at International Conference held in Bayron Bay Australia.

8. Asif, M. J., Mak, C. and R. Y. Othman, (2001). Characterization of indigenous Musa species based on Flow Cytometric Analysis of Ploidy and Nuclear DNA content. Caryologia 54 (2): 161-168.

9. Asif, M. J., Mak, C. and R. Y. Othman, (2001). In vitro zygotic embryo culture of a wild Musa acuminata ssp. malaccensis and factors affecting the germination & seedling growth. Plant Cell, Tissue and Organ Culture 67 (3): 267-270.

10. Asif, M. J., Mak, C. and R. Y. Othman, (2001). Characterization of Malaysian wild Bananas based on anthocyanins. BIOTROPIA 16:28-38

11. Asif, M. J., Mak, C. and R. Y. Othman, (2001). Fusarium wilt Race 4 studies in Musa acuminata ssp. malaccensis and further characterization of resistant and susceptible seedlings by RAPD markers. Euphytica (submitted)

12. Asif, M. J., Ng, C. Y., Fatimah, K., R. Y. Othman (2001). RAPD and STMS DNA Marker fingerprinting studies in a wild Musa acuminata ssp. malaccensis banana population (In preparation).